520 Rec'd PCT/PTO 15 OCT 1999

(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT & TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER
104560

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (if known, sec 37 C.F.R.1.5)
09/403085

OIPE JC90 OCT 15 1999 PATENT & TRADEMARK OFFICE

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR98/00772	April 16, 1998	April 16, 1997

TITLE OF INVENTION
PROCESS FOR ISOLATING A TARGET BIOLOGICAL MATERIAL, CAPTURE PHASE, DETECTION PHASE AND REAGENT

APPLICANT(S) FOR DO/EO/US
Abdelhamid ELAISSARI (Lyon, FRANCE), David DURACHER (Saint Nom La Breteche, FRANCE), Christian PICHOT (Corbas, FRANCE), Francois MALLET (Villeurbanne, FRANCE) and Armelle NOVELLI-ROUSSEAU (Seyssins, FRANCE)

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.

    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A small entity statement.

16. ☐ Other items or information:

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U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 09/403085	INTERNATIONAL APPLICATION NO. PCT/FR98/00772	ATTORNEY'S DOCKET NUMBER 104560

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO.....$840.00 International preliminary examination fee paid to USPTO (37 CFR1.482).....$670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....$760.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 96.00	$840.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	32- 20 =	12	X $ 18.00	$216.00	
Independent Claims	3- 3 =	0	X $ 78.00	$	
Multiple dependent claim(s)(if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$1,056.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28). -				$	
SUBTOTAL =				$1,056.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 month from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$1,056.00	
				Amount to be refunded	$
				Charged	$

a. ☒ Check No. 103697 in the amount of $1,056.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 15-0461. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
OLIFF & BERRIDGE, PLC
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Alexandria, Virginia 22320

Thomas Pardini

NAME: William P. Berridge
REGISTRATION NUMBER: 30,024

NAME: Thomas J. Pardini
REGISTRATION NUMBER: 30,411

(1390 Rev. 8-93)

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 09/403085	INTERNATIONAL APPLICATION NO. PCT/FR98/00772	420 Rec'd PCT/PTO 15 OCT 1999 ATTORNEY'S DOCKET NUMBER 104560

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO.....$840.00 International preliminary examination fee paid to USPTO (37 CFR1.482).....$670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....$760.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 96.00	$840.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	32- 20 =	12	X $ 18.00	$216.00	
Independent Claims	3- 3 =	0	X $ 78.00	$	
Multiple dependent claim(s)(if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$1,056.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28). -				$	
SUBTOTAL =				$1,056.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 month from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$1,056.00	
				Amount to be refunded	$
				Charged	$

a. ☒ Check No. 103697 in the amount of $1,056.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

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(1390 Rev. 8-93)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Abdelhamid ELAISSARI, David DURACHER, Christian PICHOT, Francois MALLET and Armelle NOVELLI-ROUSSEAU

Application No.: U.S. National Stage of PCT/FR98/00772

Filed: October 15, 1999 Docket No.: 104560

For: PROCESS FOR ISOLATING A TARGET BIOLOGICAL MATERIAL, CAPTURE PHASE, DETECTION PHASE AND REAGENT

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Please amend claims 5, 7-9, 11, 12, 16-18,20, 22, 24, 26, 28, 29 and 32 as follows:

Claim 5, lines 1-2, delete "or according to the combination of Claims 4 and 3".

Claim 7, lines 1-2, delete "and optionally Claim 6,".

Claim 8, line 1, delete "and/or 7".

Claim 9, line 1, delete "and/or 7".

Claim 11, line 1, change "any one of Claims 1 to 10" to --Claim 1--.

Claim 12, lines 1-2, change "any one of Claims 3 and 5 to 11" to --Claim 3--.

Claim 16, line 1, delete "or 15".

Claim 17, lines 1-2, change "any one of Claims 14 to 16" to --Claim 14--.

Claim 18, lines 1-2, change "any one of Claims 14 to 16" to --Claim 14--.

Claim 20, lines 1-2, delete "or according to the combination of Claims 3 and 19".

Claim 22, lines 1-2, delete "or according to the combination of Claims 3 and 21".

Claim 24, lines 1-2, delete "or according to the combination of Claims 3 and 23".

Claim 26, lines 1-2, delete "or according to the combination of Claims 3 and 25".

Claim 28, line 1, delete "or 3".

Claim 29, line 1, delete "or 3".

Claim 32, line 2, delete and/or use of a second polymer according to Claim 31".

REMARKS

Claims 1-32 are pending. By this Preliminary Amendment, claims 5, 7-9, 11, 12, 16-18,20, 22, 24, 26, 28, 29 and 32 are amended to eliminate multiple dependency. Prompt and favorable examination is respectfully requested.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :

U.S. National Serial No. :

Filed :

PCT International Application No. : PCT/FR98/00772

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below;

That I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the international application No. PCT/FR98/00772 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: 22 September 1999

Full name of the translator : Norval O'CONNOR
For and on behalf of RWS Group plc

Post Office Address : Europa House, Marsham Way,
Gerrards Cross, Buckinghamshire,
England.

Process for isolating a target biological material, capture phase, detection phase and reagent

The present invention relates to the isolation or detection of a biological material, referred to as the target biological material, contained in a sample, by means of a process using a capture phase, and optionally a detection phase, according to which said material is exposed to the capture phase at least, and the capture phase/target biological material complex formed is then detected, optionally with said detection phase.

In the presentation of the invention which follows, reference is made in particular to the isolation of a target protein biological material, but, needless to say, the scope of the invention should not be limited thereto.

Thus, according to the invention, the expression "biological material" means, in particular, a protein or glycoprotein material such as an antigen, a hapten, an antibody, a protein, a peptide, an enzyme or a substrate, and fragments thereof; but also a nucleic material such as a nucleic acid (DNA or RNA), a nucleic acid fragment, a probe or a primer; a hormone.

In accordance with the article by M. Kempe et al. (1), a process is known for capturing a target protein which contains polyhistidine sequences, namely RNase A, according to which the high affinity of the imidazole group of histidine for metals is used.

This process comprises the following steps:

- a capture phase is used consisting of silica particles functionalized with methacrylate groups,
- a target protein and a metal-complexing agent, namely N-(4-vinyl)benzyliminodiacetic acid (VBIDA), are placed in contact with a metal, in order to obtain a complex resulting from coordination bonding between the metal and the imidazole groups of the histidine, and coordination bonding between the metal and the carboxyl groups of VBIDA, and

- said functionalized silica particles are placed in contact with the complex formed above.

This immobilization process does not lead to optimum binding of the target protein.

Document US-A-4,246,350 describes a process for immobilizing an enzyme using a capture phase which consists of a macroporous polymer containing complexing groups linked to a transition metal. The drawback of such a capture phase results directly from the macroporous nature of the polymer. The reason for this is that, although this macroporous nature makes it possible to maximize the adsorption of the enzyme onto the capture phase, it becomes disadvantageous at the time of isolation of the enzyme using a detection phase, since the proportion of enzyme adsorbed in the polymer pores will not be accessible to said detection phase.

According to the present invention, a process is provided for isolating a target biological material, using a capture phase such that it makes it possible to optimize the binding of this material on this phase, while at the same time reducing, or even eliminating, any side reaction of adsorption of said material onto said capture phase. The interaction between the capture phase is specific, thus making it possible, during isolation, to detect the proportion of biological material effectively bound to the capture phase.

For this purpose, the process for isolating a target biological material uses a capture phase which has the following properties:

it is in microparticulate form or in linear form,

it consists of at least one first particulate or linear polymer, of hydrophilic apparent nature, and first complexing groups, linked covalently,

the first complexing groups are linked by coordination to a first transition metal,

the first transition metal is itself linked by chelation to a first biological species which is

capable of specifically recognizing the target biological material.

According to one variant of the process of the invention, the capture phase defined above comprises a marker, in order to obtain a detection phase.

According to another variant of the process, a detection phase is also used which has the following properties:

it is in microparticulate or linear form,

it consists of at least one second particulate or linear polymer, of hydrophilic apparent nature, and second complexing groups,

the second complexing groups are linked by coordination to a second transition metal,

the second transition metal is itself linked by chelation to a second biological species capable of specifically recognizing the target biological material, and a marker,

it comprises a marker.

According to the invention, the term "microparticulate" means in the form of particles not more than 10 µm in size. Preferably, they do not exceed 5 µm in size.

The first and/or second particulate or linear polymer is advantageously a hydrophilic polymer, and in particular a functionalized polymer obtained by polymerization of a water-soluble monomer, of acrylamide, of an acrylamide derivative, of methacrylamide or of a methacrylamide derivative, of at least one crosslinking agent and of at least one functional monomer.

In order to obtain this advantageous polymer, the water-soluble monomer is preferably chosen from N-isopropylacrylamide, N-ethylmethacrylamide, N-n-propylacrylamide, N-n-propylmethacrylamide, N-n-isopropylmethacrylamide, N-cyclopropylacrylamide, N,N-diethylacrylamide, N-methyl-N-isopropylacrylamide and N-methyl-N-n-propylacrylamide, the monomer preferably being N-isopropylacrylamide (NIPAM). The

functional monomer(s) preferably belong(s) to the group corresponding to formula (I) below:

$$CH_2=C(Z)-(X)_m-(R)_p-(Y)_n \quad (I)$$

in which:

Z represents H, a C1-C5 alkyl radical or a benzyl, -COOH or $-CO-NH-CH(CH_3)_2$ radical,

Y represents $-CH_2-COOH$, $-N(CH_2-COOH)_2$, $-N(CH-COOH)(CH_2-COOH)$, or $-(CH_2-CH_2-NH_2)_2$,

(CH_2-COOH)

X represents $-NH(CH_2-CH_2-)$, $-N(CH_2-CH_2-)_2$, $-N(CH_2-COOH)(CH_2-CH_2-)$, or $CH(COOH)-$,

R represents a linear hydrocarbon-based chain, optionally interrupted with at least one hetero atom such as O or N,

m and p are each an integer which, independently of each other, are equal to 0 or 1, and

n is an integer ranging between 1 and 3.

By way of example, the functional monomer is chosen from carboxylic acids, optionally containing nitrogen, itaconic acid, acrylic derivatives and methacrylic derivatives.

As stated previously, the capture phase of the invention can be in microparticulate form or in linear form.

When it is particulate, it can only consist of said particulate polymer, or alternatively it can contain a particulate support such as an organic or inorganic, hydrophilic or hydrophobic core, coated with said first polymer in particulate and/or linear form.

Said core is advantageously chosen from the group comprising polystyrene, silica and metal oxides. It can also comprise a magnetic compound.

The capture phase can also comprise a flat support, partially or totally coated with the first polymer in particulate and/or linear form.

As the examples of the present description will illustrate, the first and [lacuna] second preferred particulate polymer of the invention is poly(N-

isopropylacrylamide) (PNIPAM) comprising complexing groups derived from itaconic acid or from maleic acid-co-methyl vinyl ether.

The first and/or second transition metal is advantageously chosen from zinc, nickel, copper, cobalt, iron, magnesium, manganese, lead, palladium, platinum and gold.

According to a preferred embodiment of the process of the invention, the placing in contact of the first biological species with the capture phase and/or the placing in contact of the second biological species with the detection phase, is carried out at a pH above or equal to the isoelectric point of said first and second biological species, respectively.

The expression "biological species" means a biological material as defined above, in isolated form, and presenting, with the target biological material, an affinity to form with said material a complex of the antigen-antibody, enzyme-substrate, hormone-receptor, DNA-DNA, RNA-RNA, etc. type.

Advantageously, the first biological species is a protein. By way of example, it is the protein p24 or gp160 of HIV, for the purpose of isolating, from the serum of a patient, antibodies directed against one or other of these proteins.

The first and/or the second biological species comprises a portion capable of reacting with a transition metal, this portion preferably consisting of a histidine-rich and/or cysteine-rich region.

The sites of affinity of the biological species for the transition metal ions advantageously consist of sites rich in amino acids chosen from histidine, cysteine, tyrosine, tryptophan and phenylalinine.

The sites can be in the form of sequences of said identical or different, contiguous or non-contiguous, but neighboring amino acids.

These sites can exist naturally in the biological species, in particular when it is a protein. Alternatively, they can be "reported" beforehand into

the biological species, in the form of "tag", a definition of which is given below, according to techniques which are well known to those skilled in the art, such as the technique used for the purification of proteins by the IMAC (Immobilized Metal Ion-Affinity Chromatography) process on resins (2, 3). By way of example, such sites can be incorporated into a proteinic biological species and in particular a protein, by genetic engineering, in order to obtain recombinant proteins.

A "tag" can be defined as a reported sequence of amino acids, i.e. a sequence added to the original biological species, which is introduced at a preferred site of the original sequence, where it is exposed in a pertinent manner with respect to its chelation with the transition metal. This sequence contains amino acids chosen from those mentioned above, which are distributed inside the sequence, either contiguously (in particular two abovementioned contiguous amino acids, preferably 6 abovementioned contiguous amino acids), or with a sufficient density (in particular 25%, preferably greater than or equal to 33%). A "tag" which consists of a series of 6 contiguous histidine and/or cysteine residues will be preferred.

According to the process of the invention, a target biological material can be isolated by means of an agglutination reaction using a capture phase described above.

The marker for the detection phase is advantageously chosen from the group consisting of an enzyme, biotin, iminobiotin, a fluorescent component, a radioactive component, a chemiluminescent component, an electron-density component, a magnetic component, an antigen, a hapten and an antibody.

According to the process of the invention, a target biological material can be isolated by means of the ELISA technique using a capture phase and a detection phase, which are described above.

The invention also relates to:

- a phase for capturing a target biological material, in microparticulate or linear form and consisting of at least one first particulate or linear polymer, with hydrophilic apparent nature and first complexing groups, the latter being linked by coordination to a first transition metal, which is itself linked to a first biological species capable of recognizing the target biological material,

- a phase for detecting a target biological material, in microparticulate or linear form and consisting of at least one second particulate or linear polymer, with hydrophilic apparent nature and second complexing groups, these groups being linked by coordination to a second transition metal, which is itself linked to a second biological species capable of recognizing the target biological material, and a marker,

- a reagent for isolating a target biological material, comprising a capture phase and optionally a detection phase as defined above,

- each of the capture phase and detection phase having the properties defined above.

The characteristics and advantages of the present invention are illustrated below by Examples 1 to 5 and Figures 1 to 3 according to which:

Figure 1 represents an isotherm for the coupling of the MAVE polymer with particulate polymer poly-(St-NIPAM-AEM) particles.

Figure 2 represents the variation in the amount of protein RH24 adsorbed onto a particulate polymer poly-(St-NIPAM-MAVE) as a function of the pH and of the salinity of the medium.

Figure 3 represents the amount of protein RH24 complexed with a particulate polymer poly-(St-NIPAM-MAVE) as a function of the pH and of the salinity of the medium and for a Zn^{2+} ion concentration of the order of 0.3 M.

EXAMPLE 1: Reagents used for the preparation of the capture phase of the invention

Monomer:

- 99% styrene (Janssen Chemica, ref13 279-87), Mw=104.5 $g.mol^{-1}$

It is used after purification by distillation under vacuum.

- N-isopropylacrylamide (NIPAM) (Kodak ref. 10 982), Mw=113.16 $g.mol^{-1}$

It is recrystallized before use, as follows. It is dissolved in a hexane/toluene mixture (60/40, v/v).

Functional monomer:

- 2-aminoethylmethacrylate (AEM) chloride (Kodak ref. 18513), Mw=165.62 $g.mol^{-1}$

It is used without recrystallization.

Crosslinking agent:

- N,N-methylenebisacrylamide (MBA) (Amilabo ref. 10897), Mw=271.19 $g.mol^{-1}$

It is used without recrystallization.

Primer:

- 2,2'-azobis(2-amidinopropane) hydrochloride (V50) (Wako trade name), Mw = 271.19 $g.mol^{-1}$

V50 is recrystallized before use, as follows. The primer is dissolved in a 60/40 mixture of water and acetone. The solution is filtered under vacuum with a yield of 30%.

- Potassium persulfate (Prolabo), Mw = 270.32 $g.mol^{-1}$

It is used without recrystallization.

Complexing groups:

- itaconic acid (Aldrich), Mw = 132 $g.mol^{-1}$

It is used without recrystallization.

- Maleic anhydride-co-methyl vinyl ether (MAVE) (Polysciences)

It is used without recrystallization.

EXAMPLE 2: Synthesis of the functionalized polymer poly(N-isopropylacrylamide)-itaconic acid

4.38 g of N-isopropylacrylamide, 200 g of water, 0.37 g of MBA, 0.5 g of itaconic acid and 0.45 g

of acrylamide are placed in a 250 ml thermostatically controlled reactor. The mixture is kept stirring at 300 revolutions per minute under an atmosphere of nitrogen and at a temperature of 70°C. Potassium persulfate (0.05 g), a water-soluble primer, is introduced (dissolved in 5 g of water) into the solution at the last moment in order to start the polymerization reaction.

The polymerization reaction is continued for 5 hours under the same conditions.

The degree of conversion of the polymerization is evaluated to 98%.

The functionalized polymer obtained has the following features:

- the particle diameter, measured by dynamic light scattering, is 1500 nm,

- the assay of the surface functions, followed by conductimetry, gave 0.3 mmol/g of latex of weak acid groups (-COOH).

EXAMPLE 3: Modification of the aminohydrophilic particles by grafting the complexing linear polymer poly-MAVE

1) Synthesis of the particulate polymer poly(styrene-NIPAM)

a) Preparation of the hydrophilic particulate polymer

According to this example, the preparation consists in:

in a first stage, combining a polymer poly(St-NIPAM) containing the base monomers, i.e. styrene and NIPAM, according to a polymerization in a closed reactor, with 200 g of water, 18 g of styrene, 2 g of NIPAM and 0.2 g of V50, followed by

in a second stage, adding, to a given degree of conversion, the functional monomer (AEM), alone or in the presence of the base reagents, i.e. 5 g of NIPAM, 0 to 4% of AEM (relative to the NIPAM), 0.122 g of V50 and 0.069 g of BA.

This technique makes it possible to optimize the surface incorporation of a functional monomer. The synthesis conditions are the same as those for the polymerization in a closed reactor, i.e. constant temperature and stirring.

b) Properties of the particulate polymer obtained

The results regarding the structure of the polymer obtained, its size and its polydispersity are collated in Table 1 below.

Table 1

Name of the polymer	AEM %	(nm) 20°C (a)	(nm) 50°C (a)	Hair (nm) (b)	(nm) MET (c)	Ip (c)
DD10	0	603	364	119	288	1.012
DD15	1	421	327	47	333	1.008
DD12	2	484	334	75	302	1.004
DD11	3	358	315	21	303	1.005

(a): Diameter determined by dynamic light scattering at 20°C and at 50°C

(b): The hair corresponds to the thickness of PNIPAM at the surface of the particles

(c): Diameter and polydispersity index obtained by electron microscopy.

The degree of functionalization of the polymers obtained, expressed by the results of the assay of the amine functions present at the surface of the polymers, are given in Table 2 below.

Table 2

Name of the polymer	AEM (%) introduced	SPDP* $mmol.m^{-2}$
DD10	0	0.75
DD15	1	1.44
DD12	3	2.99
DD11	4	2.76

*: Charge density calculated using the size at 20°C determined by dynamic light scattering.

2) Grafting of poly-MAVE to the aminated particles

Complexing groups are bound covalently to the polymers obtained according to 1), these complexing groups consisting, according to the present example, of groups derived from MAVE (Maleic Anhydride-co-Methyl Vinyl Ether), which is a linear polymer.

The use of MAVE has two advantages: on the one hand, it allows, by virtue of its highly reactive anhydride functions, easy coupling with the amines present at the surface of the particulate polymer, and, on the other hand, once the coupling has been achieved, it exposes several complexing dicarboxylic functions, which will interact with a transition metal (Zn, Ni, Cu, Co, etc.).

MAVE is used as a solution in anhydrous DMSO in order to avoid hydrolysis of the anhydride functions via which the coupling reaction with the amine functions of the particulate polymers is possible. The coupling reaction should be carried out in a basic medium in order to avoid protonation of the amine functions of the polymers. The buffer used is a borate buffer of pH 8.2 and with an ionic strength of 10^{-2} M. The coupling medium should not exceed 10% by volume of DMSO.

The results, which are given in Figure 1, show a good correlation between the two analysis methods. The initial slope of the coupling isotherm shows that the reaction is complete for small amounts of MAVE introduced. The value of the plateau is 2.75 $mg.m^{-2}$ and is reached very quickly for low concentrations of MAVE.

EXAMPLE 4: Complexation of a transition metal with the polymer containing complexing groups

The introduction of a transition metal into a solution of the polymer containing complexing groups, obtained according to Example 2 or 3, should allow the binding of the metal by complexation to the particles.

This complexation takes place by means of the oxygen atoms of the anhydride functions. The presence of lone pairs on the oxygen atoms makes it possible to form coordination bonds with the transition metal.

The metal used (Zn^{2+}) is introduced into a solution of the polymer in order to obtain a concentration of metal ion solution of 10^{-4} M. The excess metal cation which is in solution is removed by successive centrifugations.

EXAMPLE 5: Complexation of the protein RH24 used as biological species to obtain a capture phase of the invention

The biological species selected for this example is the recombinant protein (referred to as RH24) modified at the N-terminal with a histidine "tag" (sequence of six contiguous histidine residues) (5). This protein has a mass of 27.10^3 g.mol^{-1} and an isoelectric point of 6.1. This modification was exploited to achieve the complexation of the protein on a particulate support, in order to obtain a capture phase of the invention.

In order to be able to determine the concentration of protein complexed on the latex, studies of adsorption of the protein were carried out in parallel.

As the state of the art shows, these are electrostatic interactions which govern the adsorption of the proteins onto a hydrophyilic polymer (6). Thus, the effect of the ionic strength and of the pH on the amount of proteins adsorbed was studied in order to determine the conditions for which the adsorption is negligible, or even nonexistent.

Figure 2 shows the adsorption of the protein RH24 onto poly(St-NIPAM-MAVE) obtained according to Example 3.

According to Figure 2, it is seen that the degree of adsorption of RH24 is highly pH-dependent.

A similar study was carried out for the complexation by varying the same parameters. Figure 3

shows the results of the complexation depending on the pH, for various ionic strengths and for constant concentrations of complexing ion (Zn^{2+}).

As seen in this figure, complexation of the protein with poly(St-NIPAM-MAVE) in the presence of zinc is little dependent on the pH, except for the low ionic strengths.

These results make it possible to determine optimum conditions for complexation at the expense of adsorption. Thus, a pH above or equal to 7 makes it possible to have virtually no adsorption while at the same time having a complexation of close to 1.5 $mg.m^{-2}$. As regards the ionic strength, this has to be minimal in order to promote the complexation.

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CLAIMS

1. Process for isolating a target biological material contained in a sample, according to which a capture phase is used, said target biological material being placed in contact with at least the capture phase, and the capture phase/target biological material complex is detected,

said process being characterized in that,

the capture phase is in microparticulate or linear form and consists of at least one first particulate or linear polymer, with a hydrophilic apparent nature and first complexing groups, these groups being linked by coordination to a first transition metal, which is itself linked to a first biological species capable of specifically recognizing the target biological material.

2. Process according to Claim 1, characterized in that the capture phase comprises a marker in order to obtain a detection phase.

3. Process according to Claim 1, characterized in that a detection phase is also used, which is in microparticulate or linear form and consists of at least one second particulate or linear polymer, of hydrophilic apparent nature, and second complexing groups, these groups being linked by coordination to a second transition metal, which is itself linked to a second biological species capable of specifically recognizing the target biological material, and a marker.

4. Process according to Claim 1 or 3, characterized in that the first and/or the second polymer is chosen from the group of hydrophilic polymers.

5. Process according to Claim 4, characterized in that the first and/or the second polymer is a functionalized polymer obtained by polymerization of a water-soluble monomer, of acrylamide, of an acrylamide derivative, of methacrylamide or of a methacrylamide

derivative, of at least one crosslinking agent and of at least one functional monomer.

6. Process according to Claim 5, characterized in that the water-soluble monomer is chosen from N-isopropylacrylamide, N-ethylmethacrylamide, N-n-propylacrylamide, N-n-propylmethacrylamide, N-n-isopropylmethacrylamide, N-cyclopropylacrylamide, N,N-diethylacrylamide, N-methyl-N-isopropylacrylamide and N-methyl-N-n-propylacrylamide, the first monomer preferably being N-isopropylacrylamide (NIPAM).

7. Process according to Claim 5, characterized in that the functional monomer corresponds to formula I below:

$$CH_2=C(Z)-(X)_m-(R)_p-(Y)_n \quad (I)$$

in which

Z represents H, a C1-C5 alkyl radical or a benzyl, -COOH or $-CO-NH-CH(CH_3)_2$ radical,

Y represents $-CH_2-COOH$, $-N(CH_2-COOH)_2$, $-N(CH-COOH)(CH_2-COOH)$, or $-N(CH_2-CH_2-NH_2)_2$,

(CH_2-COOH)

X represents $-NH(CH_2-CH_2-)$, $-N(CH_2-CH_2-)_2$, $-N(CH_2-COOH)(CH_2-CH_2-)$, or $CH(COOH)-$,

R represents a linear hydrocarbon-based chain, optionally interrupted with at least one hetero atom such as O or N,

m and p are each an integer which, independently of each other, are equal to 0 or 1, and

n is an integer ranging between 1 and 3.

8. Process according to Claim 7, characterized in that the functional monomer is chosen from carboxylic derivatives, optionally containing nitrogen, itaconic acid, acrylic derivatives and methacrylic derivatives.

9. Process according to any one of Claims 1 to 8, characterized in that the capture phase and/or the detection phase is in microparticulate form and in that the average particle size is not more than 5 µm.

10. Process according to Claim 1, characterized in that the capture phase also comprises a flat or particulate support.

11. Process according to Claim 10, characterized in that the support is particulate and consists of an organic or inorganic, hydrophilic or hydrophobic core.

12. Process according to Claim 11, characterized in that said core is chosen from the group comprising polystyrene, silica and metal oxides.

13. Process according to Claim 11 or 12, characterized in that said core also contains a magnetic compound.

14. Process according to any one of Claims 11 to 13, characterized in that said core is coated with said first polymer, this polymer being linear.

15. Process according to any one of Claims 11 to 13, characterized in that said core is coated with said polymer, said polymer being particulate.

16. Process according to Claim 1 or 3, characterized in that the first and/or the second polymer is poly(N-isopropylacrylamide) and the complexing groups are derived from itaconic acid or from maleic anhydride-co-methyl vinyl ether.

17. Process according to Claim 1 or 3, characterized in that the first and/or second transition metal is chosen from zinc, nickel, copper, cobalt, iron, magnesium, manganese, lead, palladium, platinum and gold.

18. Process according to Claim 1 or 3, characterized in that the placing in contact of the first biological species with the capture phase and/or the placing in contact of the second biological species with the detection phase is carried out at a pH above or equal to the isoelectric point of said first and second biological species, respectively.

19. Process according to Claim 1 or 3, characterized in that the first and/or the second biological species is rich in histidine and/or cysteine.

20. Process according to Claim 1 and any one of Claims 4 to 19, characterized in that an agglutination reaction is used.

21. Process according to Claim 2 or 3, characterized in that the marker for the detection phase is chosen from the group consisting of an enzyme, biotin, iminobiotin, a fluorescent component, a radioactive component, a chemiluminescent component, an electron-density component, a magnetic component, an antigen, a hapten and an antibody.

22. Process according to Claim 2 or 3 and any one of Claims 4 to 19 or 21, characterized in that the ELISA technique is used.

23. Phase for capturing a target biological material, characterized in that it is in microparticulate or linear form and consists of at least one first particulate or linear polymer, of hydrophilic apparent nature, and first complexing groups, these groups being linked by coordination to a first transition metal, which is itself linked to a first biological species capable of recognizing the target biological material.

24. Phase for detecting a target biological material, characterized in that it is in microparticulate or linear form and consists of at least one second particulate or linear polymer, of hydrophilic apparent nature, and second complexing groups, these groups being linked by coordination to a second transition metal, which is itself linked to a second biological species capable of recognizing the target biological material, and a marker.

25. Reagent for isolating a target biological material, comprising a capture phase according to Claim 23 and/or a detection phase according to Claim 24.

FIG1

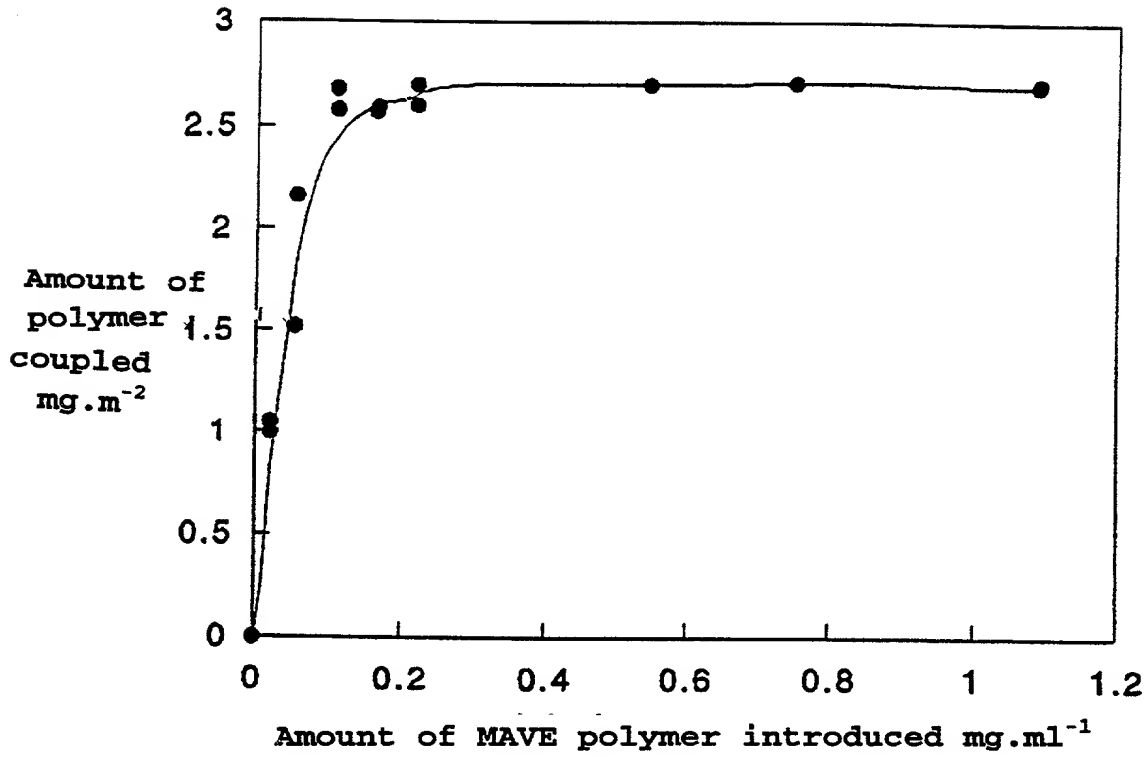

Amount of polymer coupled mg.m-2
3
2.5
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
1.2
Amount of MAVE polymer introduced mg.ml-1

FIG 2

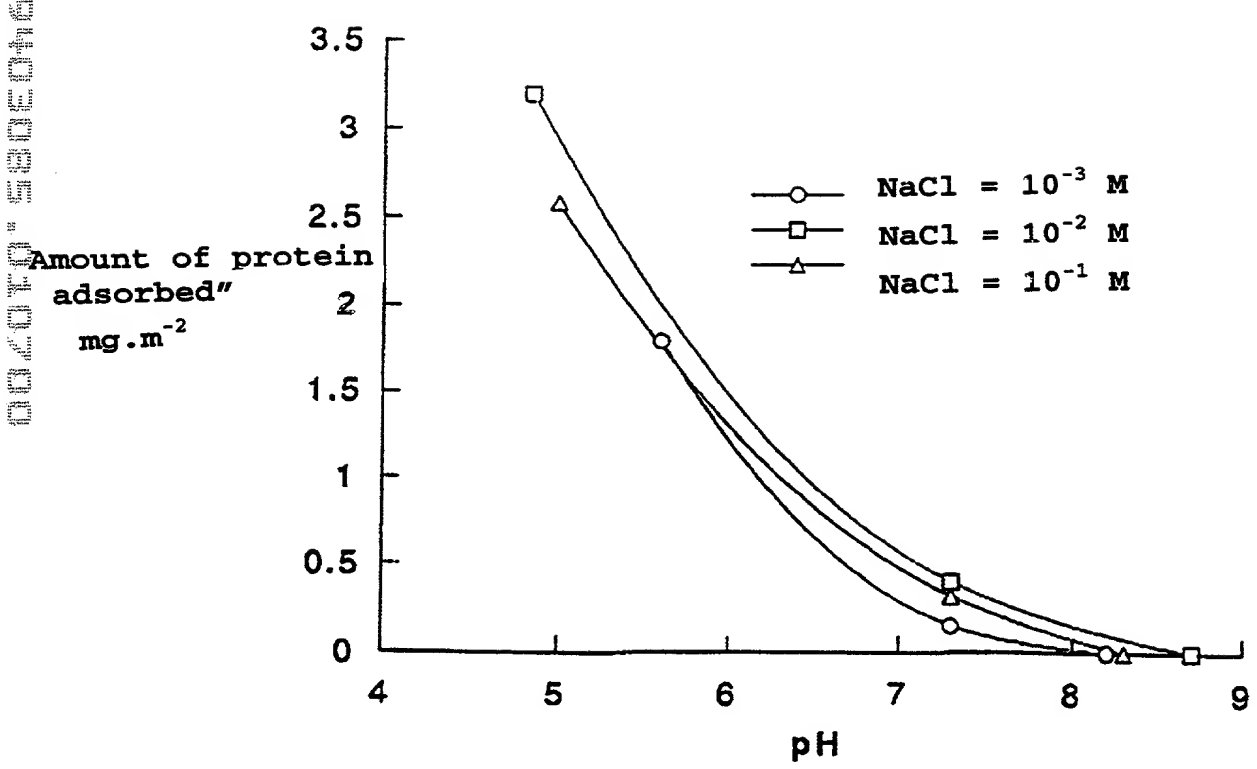
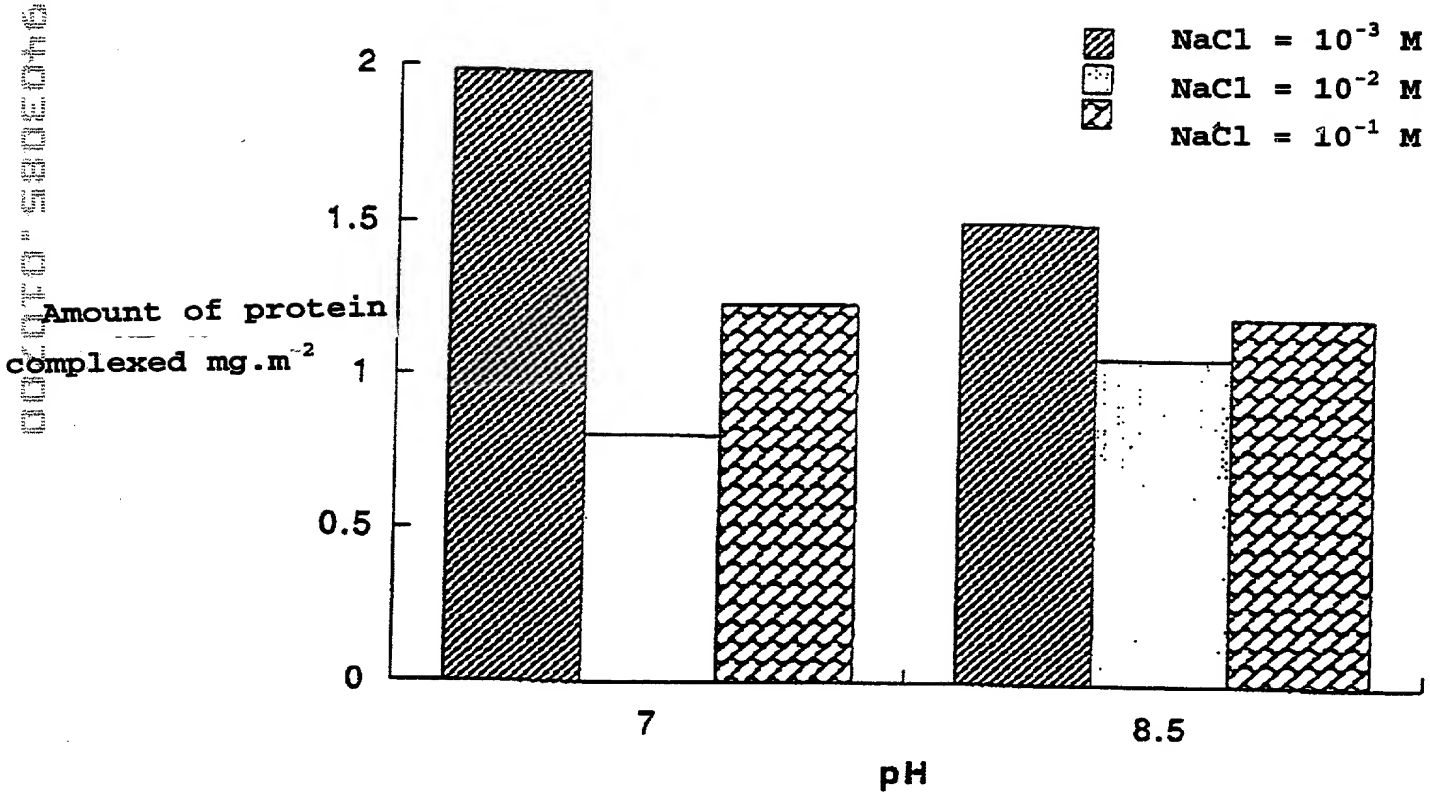

3.5
3
2.5
2
1.5
1
0.5
0
4
5
6
7
8
9
pH
"Amount of protein adsorbed"
mg.m-2
NaCl = 10-3 M
NaCl = 10-2 M
NaCl = 10-1 M

FIG 3


NaCl = 10^{-3} M
NaCl = 10^{-2} M
NaCl = 10^{-1} M
Amount of protein complexed mg.m^{-2}
2
1.5
1
0.5
0
7
8.5
pH

Docket No.: 104560

DECLARATION AND POWER OF ATTORNEY UNDER 35 USC §371(c)(4) FOR PCT APPLICATION FOR UNITED STATES PATENT

As a below named inventor, I hereby declare that:
my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled: PROCESS FOR ISOLATING A TARGET BIOLOGICAL MATERIAL, CAPTURE PHASE, DETECTION PHASE AND REAGENT described and claimed in international application number PCT/FR98/00772 filed April 16, 1998 .

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

French Patent Application No. 97/04923 filed April 16, 1997

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024; Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411; Edward P. Walker, Reg. No. 31,450; Robert A. Miller, Reg. No. 32,771; Mario A. Costantino, Reg. No. 33,565; and Caroline D. Dennison, Reg. No. 34,494.

ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE ☒
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This form may be executed only when attached to the first page of the Declaration and Power of Attorney of the application to which it pertains.